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Associations Between Acute Lipid Stress Responses and Fasting Lipid Levels 3 Years Later

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The authors assessed the association between lipid responses to acute mental stress and fasting serum lipid levels 3 years later in 199 middle-aged men and women. Total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol increased following moderately stressful behavioral tasks. LDL cholesterol, HDL cholesterol, and total:HDL ratio measured 3 years later were predicted by acute stress responses independent of gender, age, socioeconomic position, change in body mass, smoking, alcohol consumption, or hormone replacement therapy baseline lipid levels. The odds of clinically elevated cholesterol were significantly greater in the highest compared with the lowest stress tertile, independent of baseline levels and covariates. Acute lipid stress responsivity may reflect processes that contribute to the development of elevated blood cholesterol concentration.

Keywords: stress, cholesterol, mental stress testing, cardiovascular disease

There have been several studies over the past 15 years documenting the impact of acute mental stress on blood lipid concentrations. The majority of experiments have demonstrated that acute stress elicits small but significant increases in the concentration of total and low-density lipoprotein (LDL) cholesterol, with less consistent increases in high-density lipoprotein (HDL) cholesterol (Bachen, Muldoon, Matthews, & Manuck, 2002; Bacon, Ring, Lip, & Carroll, 2004; McCann et al., 1995; Muldoon et al., 1992, 1995; Stoney, Matthews, McDonald, & Johnson, 1988; Stoney, Niaura, Bausserman, & Matacin, 1999). One study involving repeated testing showed that acute stress responses were moderately stable over 16 months (Stoney, Niaura, & Bausserman, 1997).

The possible clinical significance of acute lipid stress responses is not known. There is some evidence that cholesterol responses are positively associated cross-sectionally with cardiovascular disease risk. For example, increases in total and LDL cholesterol were higher in young adults with a family history of cardiovascular disease (Stoney & Hughes, 1999). Davis (1999) reported that stress increased total and LDL cholesterol in smokers but not in nonsmokers, and associations between lipid stress responses and anger expression have been described (Finney, Stoney, & Engebretson, 2002). A comparison of premenopausal women who had undergone hysterectomy with or without the preservation of ovarian function suggested that stress-induced lipid increases were smaller if protective reproductive hormone function was maintained (Stoney, Owens, Guzick, & Matthews, 1997). However, no longitudinal studies have been published that evaluate whether acute cholesterol stress responses predict future lipid concentrations. One reason for this may be that most studies have been small scale; only one previous report has involved more than 60 participants (Stoney, Bausserman, Niaura, Marcus, and Flynn, 1999; Stoney, Niaura, et al., 1999). The power to assess longitudinal associations has therefore been limited. Additionally, most research has been carried out with healthy young men and women rather than middle-aged individuals who are closer to the age at which cardiovascular disease is typically manifest.

We carried out the present analysis with a sample of 199 middle-aged men and women in whom stress-induced changes in total cholesterol, LDL cholesterol concentration, HDL cholesterol concentration, and total:HDL ratio were assessed. The cardiovascular, cytokine, and hemostatic responses but not the lipid stress responses of this sample have previously been described (Steptoe, Feldman, et al., 2002; Steptoe, Kunz-Ebrecht, Rumley, & Lowe, 2003; Steptoe, Owen, Kunz-Ebrecht, & Mohamed-Ali, 2002). The participants completed follow-up testing 3 years after mental stress testing with measurements of fasting lipid profiles. We tested the hypothesis that stress-induced acute increases in lipid concentration would predict fasting levels 3 years later, independent of baseline lipid levels and relevant factors known to influence lipids, including age, body mass index (BMI), smoking status, alcohol consumption, and the use of hormone replacement therapy by women.

Method

Participants

Participants were 199 men and women who took part in the Whitehall II psychobiology substudy (Steptoe, Feldman, et al., 2002). The Whitehall II cohort is a sample of 10,308 London-based civil servants recruited in

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1985–1988 when participants were 35–55 years old in order to investigate demographic, psychosocial, and biological risk factors for coronary heart disease (Marmot et al., 1991). The psychobiology substudy involved 228 volunteers (123 men, 105 women) who underwent detailed laboratory investigation. Participants were of White European origin, ages 45–59 years, living in the London area, not planning to retire for at least 3 years, had no history or objective signs of coronary heart disease, and had no previous diagnosis or treatment for hypertension. Grade of employment was used as the marker of socioeconomic status, and participants were systematically recruited from higher, intermediate, and lower grades. All women in the study were peri- or postmenopausal.

The 3-year follow-up data were collected during a screening session from 209 individuals (111 men and 98 women; 92% response rate). One of the remaining 19 had died, 2 did not attend screening despite repeated requests, 4 were lost to follow-up, 3 had withdrawn from the Whitehall II study, and 9 had moved out of London and so were not invited to the screening session. Data were excluded from 5 individuals who were prescribed statins on follow-up, and cholesterol values following mental stress were not available for a further 5 participants. The study was approved by the University College London–University College London Hospitals Committee on the Ethics of Human Research.

Laboratory Mental Stress Session

The stress testing session involved assessment of cardiovascular, inflammatory, and hemostatic responses to the performance of moderately stressful behavioral tasks, as detailed elsewhere (Steptoe, Feldman, et al., 2002; Steptoe, Owen, et al., 2002). The two behavioral tasks were computerized color–word interference and mirror tracing. The color–word task involved the presentation of a series of target color words in incongruous colors. At the bottom of the computer screen were four names of colors displayed in incorrect colors, and the task was to press a computer key that corresponded to the position at the bottom of the screen of the name of the color in which the target word was printed. Mirror tracing involved the tracing with a metal stylus of a star seen in mirror image. Participants were told that the average person completed five circuits of the star in the time available and were asked to give accuracy priority over speed on both tasks.

Participants were tested individually in either the morning or afternoon in a light- and temperature-controlled laboratory. They were instructed not to have drunk tea, coffee, or caffeinated beverages or to have smoked for at least 2 hours prior to the study and not to have consumed alcohol or have exercised on the evening before or the day of testing. Body weight, height, and waist and hip circumference were measured by a research nurse using standardized methods. Information concerning smoking, alcohol consumption, and hormone replacement therapy was collected by questionnaire. A 21-gauge venous cannula was inserted, and the participant rested for 30 min, at the end of which a baseline blood sample was drawn. Participants rated their current level of stress on a 7-point scale from 1 (*low*) to 7 (*high*). The two tasks were then administered for 5 min each in random order. Following each task, ratings of stress, task involvement, difficulty, and controllability were obtained on 7-point scales. A second blood sample (stress measure) was drawn immediately after the two tasks had been completed.

Blood Assays

Blood was collected in serum gel tubes and centrifuged immediately at 2,500 rpm for 10 min at room temperature. The serum was removed and snap frozen at −70 °C until analysis. Total cholesterol was measured in a centrifugal analyzer by enzymatic colorimetric methods, and HDL cholesterol was determined after dextran sulfate-magnesium chloride precipitation of non-HDL cholesterol. LDL cholesterol was computed by using the Friedewald equation. Total cholesterol data were available for all 199 participants, and LDL and HDL cholesterol data were available for 198 people. The Friedewald equation is not accurate when triglyceride levels are high. However, only 2 individuals had plasma triglyceride concentrations above the threshold specified by the Third Report of the National Cholesterol Education Program (2002) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Removing these individuals from the LDL cholesterol analyses did not change the results. Nevertheless, because the stress session did not take place in a fasting state, we decided not to analyze triglycerides separately.

3-Year Assessment

The 3-year follow-up data were collected as part of a screening session for the full Whitehall II cohort. The interval between the stress session and follow-up averaged 3 years, 21 days ± 110 days. During this session, fasting blood samples were drawn and lipids were analyzed in the same way as for the stress session. Body weight and height were measured, and smoking and alcohol consumption were again assessed by questionnaire. Fasting lipid profiles measured 5 years before the follow-up (approximately 2 years prior to stress testing) were also available.

Statistical Methods

The background characteristics of men and women were compared by using analysis of variance for continuous variables and chi-square tests for categorical variables. Lipid responses during the stress session were analyzed with repeated measures analysis of variance with gender and grade of employment as the between-subjects factors and trial (baseline, stress) as the within-subject factor. Separate analyses were carried out for total cholesterol, LDL cholesterol, HDL cholesterol, and total:HDL ratio. Time of day of stress testing was included in preliminary models but did not affect responses and so was not incorporated into the final models. The influence of acute lipid responses to stress on fasting serum concentrations 3 years later was analyzed with multiple linear regression. Associations between independent variables were examined to ensure that multicollinearity was not present. Gender, age, hormone replacement therapy, change in BMI between stress testing (baseline) and follow-up, smoking status (coded as smoker, nonsmoker), and alcohol consumption (divided according to whether the participant drank daily or less than daily) at baseline and follow-up were included in all models. We also entered the laboratory baseline value of the lipid measure in question, together with the lipid stress response computed as the difference between stress and baseline values. Unstandardized regression coefficients (*B*) with 95% confidence intervals are presented.

Additionally, we evaluated whether lipid stress responses were associated with clinically significant elevations in fasting cholesterol 3 years later. On the basis of criteria described in the National Cholesterol Education Program (2002) Adult Treatment Panel III and the European guidelines on cardiovascular disease prevention in clinical practice (De Backer et al., 2003), we classified participants according to whether they had a fasting total cholesterol of greater than or equal to 6.2 mmol/L, a fasting LDL cholesterol of greater than or equal to 4.1 mmol/L, and a fasting total:HDL ratio of greater than or equal to 4.3 mmol/L. For each measure (total, LDL cholesterol, and total:HDL cholesterol), we divided lipid levels during the stress trial into tertiles. Logistic regression was then used to calculate the odds ratios for having 3-year fasting lipids above clinical criteria for variations in lipid levels during stress, by using the lowest stress tertile as the reference level. Odds ratios (with 95% confidence intervals) adjusted for baseline lipids, gender, age, hormone replacement, change in BMI, and smoking status and alcohol consumption both at baseline and follow-up are presented.

Results

The characteristics of the sample involved in this study are summarized in Table 1. Men and women did not differ signifi-

Table 1
Description of the Sample

	Men (n = 106)	Women (n = 93)	p
Age (years)	52.5 ± 2.6	51.8 ± 2.8	.061
Grade of employment			.97
Higher	40 (37.7%)	35 (37.6%)	
Intermediate	39 (36.8%)	33 (35.5%)	
Lower	27 (25.5%)	25 (26.9%)	
Hormone replacement therapy (%)		27 (29.0%)	
Smoking (%)			
Baseline	12 (11.4%)	6 (6.5%)	.32
Follow-up	16 (15.1%)	6 (6.5%)	.07
Alcohol consumption at least daily (%)			
Baseline	51 (48.6%)	33 (35.5%)	.084
Follow-up	44 (21.5%)	37 (39.8%)	.89
Body mass index (kg/m^2)			
Baseline	25.8 ± 3.4	25.2 ± 4.0	.23
Follow-up	26.1 ± 3.8	25.2 ± 4.3	.16
Baseline			
Total cholesterol (mmol/L)	5.49 ± 0.82	5.33 ± 0.87	.17
LDL cholesterol	3.35 ± 0.80	3.02 ± 0.76	.004
HDL cholesterol	1.43 ± 0.32	1.75 ± 0.38	.001
Total:HDL ratio	4.02 ± 1.10	3.21 ± 1.02	.001

Note. Values are means ± standard deviation or subsample, n (percentage). LDL = low-density lipoprotein; HDL = high-density lipoprotein.

cantly in age, grade of employment, smoking, alcohol consumption, or BMI. LDL cholesterol and the total:HDL ratio were significantly lower in women, $F(1, 196) = 8.57$ and 29.1, respectively, ps $< .01$, whereas HDL cholesterol concentration was higher in women than in men, $F(1, 196) = 41.0$, $p < .001$.

The repeated measures analysis of lipid values across the stress session showed main effects for trial in analyses of total cholesterol, $F(1, 197) = 76.7$, $p < .001$; LDL cholesterol, $F(1, 196) = 56.4$, $p < .001$; HDL cholesterol, $F(1, 196) = 31.6$, $p < .001$; and total:HDL ratio, $F(1, 196) = 7.92$, $p = .005$. All lipid levels increased following behavioral tasks. The gender differences in LDL cholesterol, HDL cholesterol, and total:HDL ratio observed at baseline were preserved, $F(1, 196) = 7.65$, 41.8, and 29.5, respectively, $p < .001$, but there were no interactions between trial and gender. These results are summarized in Figure 1. The increase in concentration with stress for total cholesterol averaged 0.18 ± 2.7 mmol/L, 0.13 ± 0.24 mmol/L in LDL cholesterol, 0.04 ± 0.08 mmol/L in HDL cholesterol, and 0.042 ± 1.14 mmol/L in total:HDL cholesterol ratio. It is important to note that there was wide individual variation in responses in all the lipid measures. For example, the changes in total cholesterol ranged from −0.6 to 1.6 mmol/L, LDL cholesterol ranged from −0.4 to 1.4 mmol/L, and the total:HDL ratio ranged from 1.64 to 7.10 mmol/L.

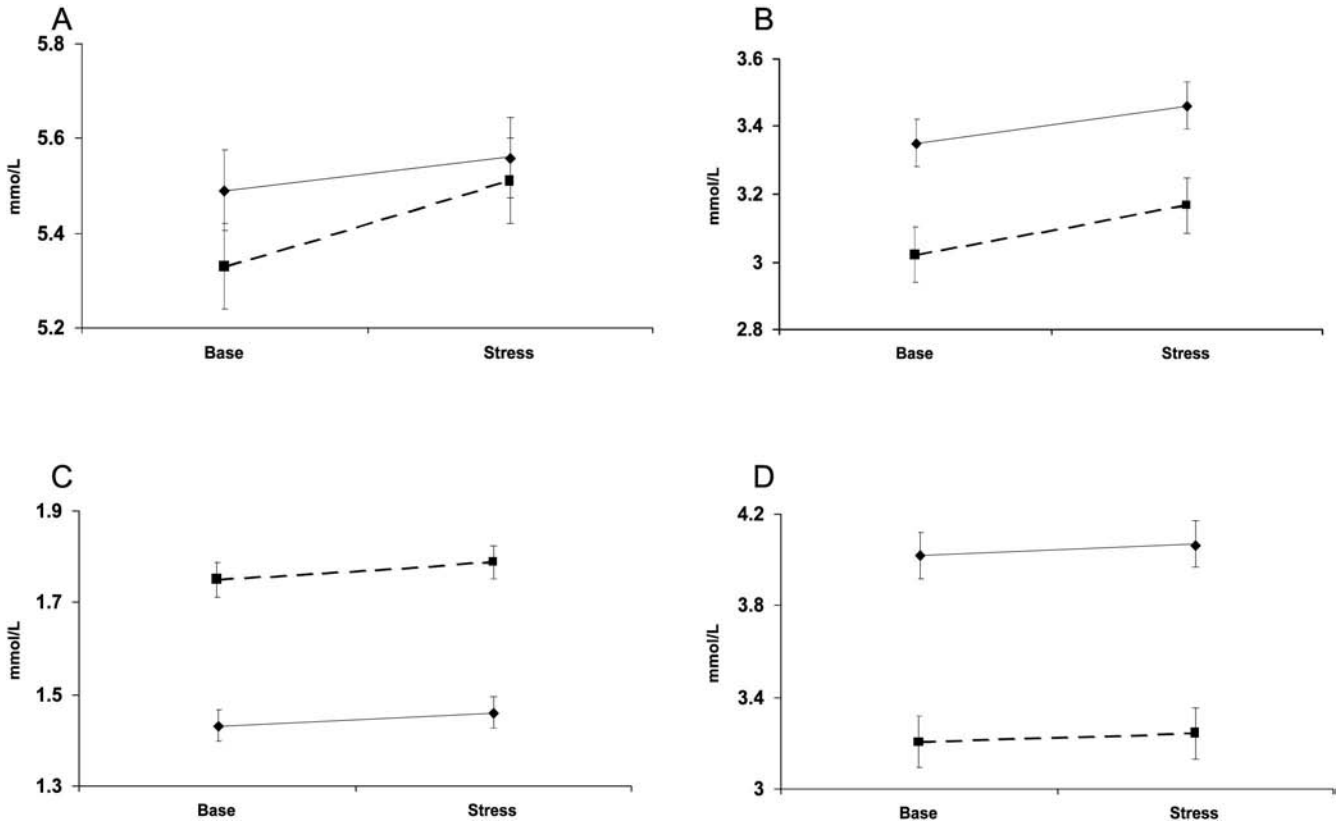

Figure 1. A: Mean levels of total cholesterol. B: Mean levels of low-density lipoprotein cholesterol. C: Mean levels of high-density lipoprotein (HDL) cholesterol. D: Mean levels of total:HDL ratio. Means are shown at baseline and following stress in men (solid lines) and in women (dashed lines). Error bars represent standard errors of the mean.

Subjective stress ratings increased from an average of 1.43 ± 0.8 at baseline to 3.99 ± 1.4 following behavioral tasks, $F(1, 195) = 620.1$, $p < .001$. This response did not vary with gender or grade of employment. Mean task ratings were 5.59 ± 1.1 for task difficulty, 5.71 ± 1.1 for task involvement, and 2.71 ± 1.1 for task controllability. There were no significant associations between subjective stress responses or task appraisals and lipid levels or responses to tasks.

Lipid Concentrations at 3-Year Follow-up

Unadjusted total serum cholesterol increased from an average of 5.41 ± 0.85 mmol/L at the baseline of the stress session to 5.82 ± 0.93 mmol/L at the clinic 3 years later, $F(1, 197) = 75.1$, $p < .001$, representing a 7.6% increase. Men and women did not differ in their responses. There was a significant upward drift in LDL cholesterol from 3.19 ± 0.79 mmol/L to 3.59 ± 0.91 mmol/L, $F(1, 196) = 79.5$, $p < .001$. HDL cholesterol also increased over time, $F(1, 196) = 76.4$, $p < .001$, and in addition there was a significant Gender × Year interaction, $F(1, 196) = 7.15$, $p = .008$. Values in men increased from 1.43 ± 0.32 mmol/L to 1.53 ± 0.37 mmol/L (an average change of 0.10 mmol/L), whereas women increased an average of 0.18 mmol/L, from 1.75 ± 0.38 mmol/L to 1.93 ± 0.43 mmol/L. The total:HDL ratio did not change on average between the stress session baseline and 3 years later (*M*s = 3.64 ± 1.1 and 3.64 ± 1.2, respectively).

Prediction of 3-Year Lipid Levels

The analyses predicting 3-year lipid levels are summarized in Table 2. Grade of employment was not included in these models, because it did not predict either lipid stress reactivity or changes over 3 years. These analyses indicated that gender was an independent predictor only of 3-year HDL cholesterol, with greater increases in women than in men ($p = .034$). Age, hormone replacement, and smoking did not predict the lipid concentrations on 3-year follow-up. The change in BMI between baseline and follow-up was positively associated with 3-year total, LDL cholesterol, and total:HDL cholesterol ratio (all *p*s $< .001$) but was negatively related to HDL cholesterol ($p < .001$). Alcohol consumption at follow-up was associated with the increase in HDL cholesterol ($p = .032$). For all lipid variables, baseline levels during the stress session were strong and consistent predictors of fasting levels 3 years later ($p < .001$). Additionally, stress responses in LDL cholesterol ($p = .030$), HDL cholesterol ($p < .001$), and total:HDL ratio ($p = .003$) were independent predictors of 3-year fasting serum levels. In each case, the greater the stress response, the higher the fasting concentration 3 years later, independent of other factors. The independent association between total cholesterol stress responses and fasting levels 3 years later was not significant ($p = .066$). The variance accounted for by these models ranged from 59% (total cholesterol) to 80% (HDL cholesterol). There was no significant cross-reactivity; thus, HDL cholesterol stress reactions did not predict 3-year total cholesterol, stress reactions in total cholesterol did not predict 3-year LDL cholesterol, and so forth.

Prediction of Clinically Significant 3-Year Lipid Levels

We assessed whether total cholesterol stress reactions independently predicted total cholesterol levels greater than or equal to 6.2 mmol/L 3 years later by using logistic regression. Stress levels of total cholesterol were divided into tertiles. We found that 15.9% of individuals with total cholesterol in the lowest stress tertile had values above threshold 3 years later, compared with 56.1% of those in the highest stress total cholesterol tertile, after adjusting for baseline total cholesterol and other covariates (see Table 3). The adjusted odds of having a fasting total cholesterol greater than or equal to 6.2 mmol/L 3 years after stress testing were 13.1 (confidence interval = 1.20 to 142.5, $p = .035$) for participants in the highest compared with the lowest stress total cholesterol tertile. When fasting total cholesterol obtained 2 years earlier was substituted for the stress session baseline as a covariate in these analyses, the adjusted proportions with levels above threshold at follow-up were similar to those in Table 3 (20.8%, 22.1%, and 51.5% for

Table 2
Predictors of 3-Year Follow-Up Fasting Lipid Concentrations

	Total cholesterol		LDL cholesterol		HDL cholesterol		Total:HDL cholesterol	
Predictor	*B* (95% CI)	*p*	*B* (95% CI)	*p*	*B* (95% CI)	*p*	*B* (95% CI)	*p*
Gender[a]	0.19 (−.01, .39)	.062	−0.05 (−.14, .23)	.64	0.08 (.01, .15)	.034	−0.13 (−.88,2.60)	.21
Age	0.02 (−.01, .06)	.18	0.01 (−.02, .04)	.58	0.01 (−.01, .02)	.23	−0.01 (−.32, .07)	.98
Hormone replacement	−0.14 (−.42, .14)	.32	−0.17 (−.44, .09)	.20	.01 (−.09, .10)	.85	−0.05 (−.31, .21)	.70
Body mass index change (baseline to follow-up)	0.13 (.07, .19)	.001	0.13 (.08, .19)	.001	−0.04 (−.06,−.02)	.001	0.19 (0.13, .24)	.001
Smoking status—baseline	0.20 (−.29, .69)	.41	0.33 (−.13, .79)	.16	0.08 (−.08, .24)	.33	−0.03 (−.48, .42)	.90
Smoking status—follow-up	−0.11 (−.56, .35)	.64	−0.06 (−.49, .36)	.76	−0.09 (−.24, .06)	.23	0.16 (−.26, .58)	.46
Alcohol consumption—baseline	0.13 (−.17, .43)	.39	0.233 (−.05, .51)	.11	−0.01 (−.20, .01)	.054	0.20 (−.08, .48)	.17
Alcohol consumption—follow-up	−0.12 (−.42, .19)	.45	−0.25 (−.54, .03)	.08	0.11 (.01, .22)	.032	−0.26 (−.54, .03)	.08
Baseline lipid level[b]	0.81 (.71, .91)	.001	0.83 (.72, .93)	.001	0.93 (.86, 1.02)	.001	0.81 (.73, .89)	.001
Lipid stress response[c]	0.30 (−.02, .61)	.066	0.38 (.04, .72)	.030	0.61 (.25, .98)	.001	0.58 (.19, .96)	.003
R^2	.59	.001	.62	.001	.80	.001	.77	.001

Note. LDL = low-density lipoprotein; HDL = high-density lipoprotein; CI = confidence interval.
[a] Coded 1 = men, 2 = women. [b] Lipid measure appropriate for the column. [c] Stress − baseline change score of lipid measure appropriate for the column.

Table 3
Prediction of Clinically Significant 3-Year Lipid Concentrations

	Total cholesterol ≥ 6.2 mmol/L		LDL cholesterol ≥ 4.1 mmol/L		Total:HDL ratio ≥ 4.3 mmol/L	
Lipid stress tertile[a]	Percentage with levels above threshold	Adjusted odds for levels above threshold (95% CI)[b]	Percentage with levels above threshold	Adjusted odds for levels above threshold (95% CI)[c]	Percentage with levels above threshold	Adjusted odds for levels above threshold (95% CI)[d]
Lowest	15.9%[b]	1	23.5%[c]	1	18.2%[d]	1
Middle	22.7%	3.58 (0.61, 21.0)	27.6%	3.41 (0.60, 19.3)	27.7%	3.16 (0.70, 14.6)
Highest	56.1%	13.1 (1.20, 142.5)	40.3%	3.98 (0.41, 38.8)	31.7%	4.76 (1.20, 18.9)

Note. LDL = low-density lipoprotein; HDL = high-density lipoprotein; CI = confidence interval; BMI = body mass index.
[a] Lipid measure appropriate for the column. [b] Adjusted for gender, age, hormone replacement therapy, change in BMI, smoking status, and alcohol consumption at baseline and follow-up and for baseline total cholesterol. [c] Adjusted for gender, age, hormone replacement therapy, change in BMI, smoking status, and alcohol consumption at baseline and follow-up and for baseline LDL cholesterol. [d] Adjusted for gender, age, hormone replacement therapy, change in BMI, smoking status, and alcohol consumption at baseline and follow-up and for baseline total:HDL ratio.

individuals in the lowest, middle, and highest stress total cholesterol tertiles, respectively).

A comparable analysis was carried out for LDL cholesterol greater than or equal to 4.1 mmol/L. Although the pattern of results was similar (see Table 3), effects were not significant. However, significant effects did emerge in the analysis of the proportion of participants with total:HDL ratios above threshold (4.3) at the 3-year follow-up. As can be seen in Table 3, 18.2% of those in the lowest total:HDL ratio stress tertile had 3-year values above threshold, compared with 31.7% of individuals in the highest total:HDL ratio stress tertile, after adjustment for baseline total:HDL ratio level and other covariates. The adjusted odds of a fasting total:HDL ratio above threshold were 4.76 (confidence interval = 1.20 to 18.9, $p = .027$), for participants in the highest compared with the lowest stress total:HDL ratio tertile. When the fasting total:HDL ratio obtained 2 years before stress testing was substituted for the stress session baseline ratio as a covariate, the adjusted proportion with total:HDL ratios above threshold on 3-year follow-up were 21.4%, 26.3%, and 31.7% for the lowest, middle, and highest stress total:HDL ratio tertiles, respectively, and so were similar to the pattern described in Table 3.

Discussion

This study was intended to advance the investigation of acute lipid stress responses by examining the prognostic significance of individual differences in acute responsivity. We found in this sample of healthy middle-aged men and women that behavioral tasks stimulated increases in total cholesterol, LDL cholesterol, and HDL cholesterol. Individual differences in LDL and HDL cholesterol stress responses and in total:HDL ratio responses predicted 3-year fasting HDL cholesterol and total:HDL ratio, respectively, independent of baseline serum cholesterol levels, gender, age, hormone replacement, change in BMI, smoking, and alcohol consumption at both time points. The odds of clinically elevated fasting total cholesterol level at 3 years were substantially greater among individuals with post-stress lipid concentrations in the highest versus lowest tertile (odds ratio = 13.1), although with large confidence intervals. Similarly, the odds of a clinically significant total:HDL ratio 3 years after stress testing were raised in higher stress responders, independent of baseline total:HDL ratio and other covariates.

The unadjusted increases in total cholesterol, LDL cholesterol, and HDL cholesterol in this study averaged 3.2%, 4.2%, and 2.5%, respectively. There was also a small rise in the total:HDL cholesterol ratio, as a result of the relatively greater increase in total cholesterol compared with HDL cholesterol concentrations. These increases are broadly comparable to those previously observed with a range of behavioral tasks and study groups. An analysis of 10 previous studies showed that total cholesterol increased 2.73% on average (ranging from −1.2% to 5.5%), LDL cholesterol increased by 2.97% (ranging from −1.2% to 6.0%), and HDL cholesterol increased by 2.44% (ranging from −0.7% to 5.1%) (Bachen et al., 2002; Bacon et al., 2004; Davis, 1999; McCann et al., 1995; Muldoon et al., 1995; Patterson, Matthews, Allen, & Owens, 1995; Stoney & Hughes, 1999; Stoney et al., 1988; Stoney, Niaura, & Bausserman, 1997; Stoney, Niaura, et al., 1999). Some of the variations between studies are likely due to the intensity of the stressor applied, but the age of participants may also be relevant. The increase that we observed in LDL cholesterol was somewhat above average for this literature. Most researchers have studied young men and women, and larger acute lipid responses have previously been reported in middle-aged samples (McCann et al., 1995). Bacon et al. (2004) recently described rather modest increases in total cholesterol (1.2%) and LDL cholesterol (1.9%) in 51 patients with suspected coronary artery disease admitted for angiography or angioplasty. It is not clear whether the small responses in that study were due to the nature of the behavioral challenge, the characteristics of the sample, or the fact that half the participants were taking statins. The tasks in the present study elicited significant increases in subjective stress ratings, and they were appraised as difficult, involving, and relatively uncontrollable. It is interesting to note that there were no significant associations between subjective and lipid responses.

There were no gender differences in cholesterol stress responses. Previous literature has shown less consistent HDL cholesterol increases with acute stress in men than in women, and two studies exclusively or predominantly involving men reported no increase in HDL cholesterol at all (Bacon et al., 2004; Stoney, Bausserman, et al., 1999). We were not able to replicate the

significantly larger stress-induced increase in LDL cholesterol in young men than in women described by Stoney et al. (1988).

An issue that has been intensively investigated in this literature is whether the changes in concentration following stress reflect increases in lipid biosynthesis or release into the circulation or are secondary to reductions in plasma volume leading to increased concentration of circulating proteins. A number of studies have found that after correcting for changes in hemoconcentration, the increases in lipid levels are no longer significant (Bachen et al., 2002; Bacon et al., 2004; McCann et al., 1995; Muldoon et al., 1995; Patterson, Gottdiener, Hecht, Vargot, & Krantz, 1993, Patterson et al., 1995). In other experiments, changes have remained significant after adjusting for hemoconcentration (Stoney, Bausserman, et al., 1999; Stoney, Niaura, & Bausserman, 1997). In the present study, we measured hematocrit (data not shown) but not hemoglobin, so we could not compute plasma volume. It is possible that if this factor had been included in the analysis, the increases in total cholesterol, LDL cholesterol, and HDL cholesterol would no longer have been significant.

The mechanisms underlying the associations between acute stress responses and subsequent elevations in fasting serum lipids are uncertain. One possibility is that individual differences in stress-induced lipolysis are responsible (Brindley, McCann, Niaura, Stoney, & Suarez, 1993). Mammals have evolved so that in times of stress, extra energy is supplied to the blood in the form of metabolic fuels—namely, fatty acids and glucose. Catecholamines stimulate lipolysis in adipose tissue, through activation of hormone-sensitive lipase, leading to the breakdown of triacylglycerols into fatty acids and glycerol. This effect is sensitized by cortisol (Brindley et al., 1993). Increased levels of fatty acids and cortisol lead to insulin insensitivity in tissues and promote increased triacylglycerol synthesis and apolipoprotein B secretion by the liver. These combined effects result in increased hepatic production and secretion of very low-density lipoprotein, which is ultimately converted to LDL, the principal carrier of cholesterol in the blood.

A second possibility that has been proposed by Stoney, Hughes, Kuntz, West, and Thornton (2002) is that there may be alterations in lipid clearance following stress. This was indirectly tested by measuring the clearance of an intravenously administered fat emulsion with and without concurrent stress. The clearance rate for the exogenous fat load was found to be significantly reduced by stress. Reduced lipid clearance could be due to reduced activity of lipoprotein lipase, the rate-limiting enzyme for catabolism of triglyceride-rich proteins. Preliminary data from Stoney et al. (2002) suggest that lipoprotein lipase activity is diminished during acute psychological stress. Accordingly, epinephrine has been shown to inhibit lipoprotein lipase secretion at a posttranslational level in vitro (Yukht, Davis, Ong, Ranganathan, & Kern, 1995). If a similar effect occurred in vivo, it could affect clearance of triglyceride-rich proteins, resulting in their accumulation in the blood stream. However, against a β-adrenergic-driven mechanism is the observation that β-blockade had no effect on stress-induced increases in total LDL or HDL cholesterol (Bachen et al., 2002).

Another potential mechanism is stress-induced down regulation of the hepatic LDL receptor. LDL is normally cleared from the blood through binding to this receptor. LDL receptor expression is stimulated by insulin and inhibited by cortisol (Brindley & Salter, 1991; Salter, Fisher, & Brindley, 1987). Stress-induced insulin resistance, together with increased production of cortisol, could delay LDL clearance by inhibiting expression of its receptor.

It is also conceivable that both acute lipid responses to stress and subsequent fasting lipid concentrations are consequences or correlates of other underlying processes. We were able to eliminate body mass, smoking, and alcohol consumption as cofactors, and differences in subjective stress did not correlate with lipid responses. We did not assess diet, but it is unlikely that dietary patterns contribute to individual differences in acute lipid stress responses. Notably, lipid metabolism in atherogenesis is intimately linked with inflammatory processes. Modified lipids are incorporated into atheroma and induce the expression of adhesion molecules, proinflammatory cytokines, and other mediators of inflammation (Libby, Ridker, & Maseri, 2002). Cardiovascular event-free survival was predicted by the combination of inflammation (indexed by C-reactive protein) and LDL cholesterol in the Women's Health Study (Ridker, Rifai, Rose, Buring, & Cook, 2002). Others have shown that acute stress stimulates increases in plasma interleukin 6, tumor necrosis factor α, and C-reactive protein, and it is possible that these inflammatory factors influence stress-induced increases in lipids (Steptoe, Owen, et al., 2002; Steptoe, Strike, et al., 2003). The cellular source of stress-induced increases in inflammatory cytokines has yet to be identified; however, one potential source that has been proposed is adipose tissue. Interleukin-6 is produced by adipose tissue and has recently been shown to be a potent modulator of fat metabolism in humans, stimulating both lipolysis and fat oxidation (van Hall et al., 2003).

A limitation in this study is that we did not obtain a fasting blood sample for assessing lipid profiles at the time of mental stress testing. We therefore used baseline levels from the stress session rather than fasting values as the covariates in analyses. Nonetheless, because the study was carried out within the context of a continuing prospective epidemiological study, we were able to use fasting lipids that had been recorded approximately 2 years prior to stress testing and 5 years before the follow-up data analyzed in this article. When these values were substituted for the stress session baseline levels, the associations between stress responses and subsequent fasting concentrations persisted. However, these earlier fasting values were not an ideal proxy for concurrent fasting levels, because changes in fasting lipid levels could have taken place in the interval between these earlier fasting samples and stress testing, rather than between stress testing and the 3-year follow-up. The baseline levels recorded during the stress session are therefore more reliable indicators for the prospective analyses.

Other limitations to the study should be acknowledged. Although the sample size was larger than previously assessed in this literature, participation was restricted to White, middle-aged working men and women, so results may not generalize to other populations. We measured lipids only before and immediately after mental stress testing. In allied analyses of the Whitehall psychobiology substudy, variations have been documented in the rate of post-stress recovery that relate to socioeconomic status and may be significant for cardiovascular health (Steptoe, Feldman, et al., 2002; Steptoe, Kunz-Ebrecht, et al., 2003). Similar patterns could not be assessed in the present work. The Friedewald equation for calculating LDL cholesterol was derived for the analysis of fasting lipids. We reasoned that the absence of high triglyceride levels meant that the equation could be used on our nonfasting samples, but more direct measures would have been desirable.

There are in addition other factors, such as dietary choices, that might have influenced changes in lipid concentrations over the 3-year period that were not measured. Nevertheless, the observation that stress-induced lipid responses were associated with later concentrations despite such potential influences is encouraging. It raises the intriguing possibility that even though lipid responses to acute stress are small, individual differences in response may be relevant to future fasting lipid concentrations. Because lipid levels are consistently associated with future coronary heart disease, this stress-related mechanism may be clinically significant.

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